

1 **No evidence that mate choice in humans is dependent on the MHC**

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11 **Short title:**

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29

30 **Abstract**

31 A long-standing hypothesis in biology proposes that various species select mates with a
32 major histocompatibility complex (MHC) composition divergent from their own, so as to
33 improve immune response in offspring. However, human and animal studies
34 investigating this mate selection hypothesis have returned inconsistent results. Here, we
35 analyze 239 mate-pairs of Dutch ancestry, all with whole-genome sequence data
36 collected by the Genome of the Netherlands project, to investigate whether mate
37 selection in humans is MHC dependent. We find no evidence for MHC-mediated mate
38 selection in this sample (with an average MHC genetic similarity in mate pairs (Q_c) =
39 0.829; permutation-based $p = 0.703$). Limiting the analysis to only common variation or
40 considering the extended MHC region does not change our findings ($Q_c = 0.671$, $p =$
41 0.513; and $Q_c = 0.844$, $p = 0.696$, respectively). We demonstrate that the MHC in
42 mate-pairs is no more genetically dissimilar (on average) than a pair of two randomly
43 selected individuals, and conclude that there is no evidence to suggest that mate choice
44 is influenced by genetic variation in the MHC.

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55 **Author summary**

56 Studies within various animal species have shown that the genetic content of the major
57 histocompatibility complex (MHC) can influence mate choice. Such mate selection would
58 be advantageous, as mating between individuals with different alleles across MHC genes
59 would produce offspring with a more diverse MHC and therefore possess improved
60 immune response to various pathogens. Studies of the influence on the MHC in human
61 mate selection have been far less conclusive. Two studies of MHC-dependent mate
62 selection performed on SNP data collected as part of the HapMap Consortium returned
63 conflicting results: the first study reported significantly different MHC variation between
64 mate pairs, and the second report refuted this claim. Here, we analyze a dataset
65 comprised of 239 whole-genome sequenced Dutch mate pairs, a sample set an order of
66 magnitude larger than the HapMap data and containing denser characterization of
67 genetic variation. We find no evidence that the MHC influences mate selection in our
68 population, and we show that this finding is robust to potential confounding factors and
69 the types and frequencies of genetic variants analysed.

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71

72 **Introduction**

73

74 The extended major histocompatibility complex (MHC) spans an approximately 7-
75 megabase region on chromosome 6 in humans. The region codes for a series of proteins
76 critical to acquired immune function as well as olfactory genes [1]. Additionally, the MHC
77 contains extensive genetic diversity [2,3], much more so than other regions of the
78 genome; within the human population, the MHC contains thousands of different alleles
79 and haplotypic combinations spanning the frequency spectrum. Genome-wide
80 association studies (GWAS) have identified a plethora of genetic variants in the region
81 associated to a host of diseases [4], both with and without previously-described roles for
82 immune function [5–10].

83

84 Some biological studies have proposed that, beyond the direct role in immune function,
85 the MHC may influence mate selection in vertebrate species. Increased MHC diversity is
86 evolutionarily advantageous, as it improves immune response to a wider range of
87 pathogens [11,12]. A number of studies in (non-human) animals indicate that some
88 species of mice, birds, and fish, preferentially mate to maintain or increase MHC diversity
89 [13–17]. For example, studies in sticklebacks [18] indicate that MHC-based mate
90 selection helps to optimize copy number of particular MHC loci between mates. In mice,
91 increased MHC dissimilarity between mates increases diversity of amino acid
92 substitutions within binding-pockets of specific HLA molecules [19,20]. Many of these
93 studies suggest that the observed MHC-dependent mate selection is mediated by the
94 olfactory system, either through detectable residues that mates can smell [21], or
95 because olfactory receptor genes are often found to cluster in close genomic proximity to
96 the MHC [3].

97

98 Evidence for MHC-dependent mate selection in humans is far less conclusive. A study of
99 411 couples from the Hutterite population, a population isolate in North America,

100 performed HLA typing across all couples and found that couples had more MHC diversity
101 than expected under random mating [22]. Two additional studies, of 200 Amerindian
102 couples [23] and 450 Japanese couples [24], respectively, concluded that the differences
103 between the HLA-types of real couples were not significantly more different than the HLA
104 types of random pairs of individuals. Finally, additional work has investigated whether
105 the remnants of degraded HLA proteins end up in sweat, urine or saliva and can
106 therefore be detected by potential mates through scent. To test the hypothesis that
107 MHC-dependent mate selection in humans is mediated through olfactory processes,
108 researchers have performed so-called 'sweaty t-shirt' experiments, and shown that
109 females indicate an odor preference towards men that carry divergent HLA alleles
110 relative to their own [25,26].

111
112 Studies of genetic variation (beyond the classical HLA types) in humans have sought to
113 provide clarity as to whether humans do indeed select mates, at least in part, such that
114 diversity across the MHC increases in offspring. An initial analysis of array-based SNP
115 genotyping data (variation with minor allele frequency (MAF) > 5%) assembled by the
116 HapMap 2 Consortium [27] examined 30 European-ancestry mate pairs and 30 African-
117 ancestry mate pairs and reported evidence of dissimilar MHC variation in couples of
118 European descent ($p = 0.015$) [17]. Conversely, no such effect was observed in the
119 African-ancestry sample ($p = 0.23$) [17]. A subsequent analysis in the same Hapmap
120 Phase 2 European-ancestry data, but including an additional 24 European-ancestry
121 mate-pairs genotyped as part of HapMap Phase 3 [28], failed to replicate the initial
122 finding [29]. This second analysis demonstrated that the low sample size of the initial
123 analysis (making the study sensitive to small changes in parameter choices) and failure
124 to correct for multiple testing explained the initial report. Neither analysis of the 24 new
125 mate-pairs nor joint analysis of all 54 available European-ancestry mate pairs revealed
126 increased MHC dissimilarity in mates ($p = 0.351$ and $p = 0.143$, respectively).

127

128 Here, we aim to test whether human mate pairs are indeed more dissimilar across the
129 MHC, using a sample set that represents an order-of-magnitude increase over the initial
130 reports. Specifically, we test the hypothesis that MHC variation is discordant between
131 couples by analyzing a dataset of 239 unrelated Dutch mate pairs, whole-genome
132 sequenced as part of the Genome of the Netherlands (GoNL) project [30]. The density
133 and resolution of the whole-genome sequence data allow us to test for discordant MHC
134 variation in mate pairs with respect to (a) common variation only (MAF > 1%); (b) the
135 full frequency spectrum of genetic variants, including single nucleotide variants and short
136 insertions and deletions; and (c) imputed amino acids and human leukocyte antigen
137 (HLA) types within the MHC [31].

138 **Results**

139

140 **Reproducing the initial HapMap analysis**

141

142 We first sought to reproduce the finding of MHC-dependent mate selection in humans
143 reported from an analysis of common variation in the Hapmap Phase 2 data [17], with
144 the goal of not only replicating results but also aligning methodologies. The previous
145 analysis used 30 trios of Northern- and Western-European ancestry living in Utah, USA
146 (called the CEU sample) and 30 trios collected from the Yoruba population in Ibadan,
147 Nigeria (called the YRI sample) [27,32,33] to evaluate MHC genetic dissimilarity in mate
148 pairs. After reproducing the quality control procedures from the initial analysis as closely
149 as possible (**Materials and Methods**), 27 CEU and 27 YRI mate-pairs remained for
150 analysis (**Table 1**).

151

152 We used the same measure for genetic similarity between two individuals as defined in
153 the initial report: Q_c , defined as 'the proportion of identical genotypes (at variant
154 positions)' [17] between mate pairs (**Materials and Methods**). We compared the
155 average similarity across real couples to the average similarity across randomly
156 generated mate pairs (created by randomly drawing a male and a female from the
157 sample) and obtained results that are close, but not identical to, the initial report
158 (**Figure 1**). We calculated the difference between average genetic similarity across all
159 true mate pairs and average genetic similarity across permuted mate pairs (i.e., average
160 Q_c across a null distribution; **Figure 1**) to explicitly quantify how genetic similarity in true mate
161 pairs deviates from the null distribution. We call this metric ΔQ_c . We found that the CEU mate pairs
162 demonstrated nominally-significant ($p < 0.05$) genetic dissimilarity across the MHC compared to
163 permuted mate pairs ($\Delta Q_c = -0.013$, 2-sided $p = 0.023$), while mate-pairs in the YRI samples

164 indicated no such relationship ($\Delta Q_c = 0.003$, 2-sided $p = 0.442$). Genome-wide, CEU mate pairs
165 showed no pattern of genetic similarity or dissimilarity ($\Delta Q_c = -0.008$, 2-sided $p = 0.100$)
166 while YRI mate-pairs showed a pattern of genome-wide similarity (average $Q_c = 0.011$,
167 2-sided $p < 10^{-6}$), consistent with the original report [17].

168

169 **Testing MHC-specific genetic dissimilarity in the Genome of the Netherlands**

170

171 Next, we sought to test if there was evidence for MHC-dependent mate selection in mate
172 pairs collected as part of the Genome of the Netherlands (GoNL) project [30]. GoNL is
173 comprised of Dutch-ancestry trios (confirmed by principal component analysis [30])
174 drawn from 11 of the 12 provinces of the Netherlands and whole-genome sequenced at
175 $\sim 14x$ average coverage on the Illumina HiSeq 2000 [30]. After data quality control and
176 processing in the original project [30], the GoNL dataset contained 248 mate pairs.
177 Because relatedness is a primary confounder for genetic similarity estimations, we
178 calculated sample relatedness in Plink [34] and removed an additional 9 mate pairs with
179 $\pi\text{-hat} > 0.03125$ (a threshold corresponding to 5th-degree relatedness; **Materials and**
180 **Methods**). After this additional quality control, 239 mate pairs remained for analysis.
181 We analyzed the GoNL data (<http://www.nlgenome.nl/>, see Code and Data Release in
182 **Materials and Methods**) from Release 5 of the project, which includes single-nucleotide
183 variants (SNVs) and short ($< 20\text{bp}$) insertions and deletions (indels; **Table 1**).

184

185 To test for MHC-dependent mate selection in GoNL, we extracted the MHC (chromosome
186 6, 28.7 - 33.3Mb on build hg19), calculated Q_c across all true GoNL mate pairs, and
187 performed the same permutation scheme as in the HapMap analysis, randomizing the
188 mate pairs, recalculating the average Q_c across these randomly-constructed pairs, and
189 finally calculating ΔQ_c . All p -values are 1-sided, testing the hypothesis of genetic
190 dissimilarity, unless otherwise stated. Our results showed no evidence for MHC-
191 dependent mate selection ($\Delta Q_c = 0.0005$, permutation $p = 0.702$, **Figure 2**). Restricting

192 our analyses to common- and low-frequency SNPs (MAF > 0.5%) or common SNPs only
193 (MAF > 5%) did not change our results (**Table 1, Supplementary Figures 1 and 2**),
194 nor did restricting the analysis specifically to the ~2M common SNPs genotyped in
195 HapMap 2 or including the set of ~2M indels sequenced in GoNL into the analysis (**Table**
196 **1 and Supplementary Figure 3**). To test the hypothesis that MHC mating is mediated
197 through olfactory sensory pathways, as hypothesized previously [25,26], we performed
198 the same analysis using an extended definition of the MHC (26.6Mb - 33.3Mb on hg19),
199 which includes a dense cluster of 36 olfactory receptor genes upstream of the HLA Class
200 I region [3]. We observed no statistically significant effect (**Table 1**, and
201 **Supplementary Figures 4 and 5**).

202

203 Though the Netherlands is geographically small and densely populated, both common
204 and rare variation in the GoNL data indicate geographic clustering [30,35–37]. We
205 therefore investigated whether population stratification may explain the discordance
206 between our results and the previous report of MHC-dependent mate selection in
207 humans [17]. We performed genetic similarity analyses in the samples split into three
208 geographic regions (“north,” “middle,” and “south” as determined by an identity-by-
209 descent analysis [30]), as well as by province. Subsetting by region or province revealed
210 no evidence for subpopulation-specific MHC-dependent mate selection (**Figure 2**).
211 Additionally, accounting for sample ancestry using principal components (**Materials and**
212 **Methods**) left our results unchanged ($p = 0.78$).

213

214 Lastly, we used SNP2HLA [31] to impute 2- and 4-digit HLA alleles, amino acids and
215 SNPs (**Materials and Methods**) into the GoNL samples as a means of evaluating
216 genetic (dis)similarity across imputed HLA types. Given that the dosages output from
217 SNP2HLA are phased, we used the Pearson’s correlation (r) across the imputed allele
218 dosages to calculate genetic similarity (instead of the Q_c metric). We found no evidence
219 for MHC-dependent mate selection either across all imputed markers ($p = 0.48$, **Table**
220 **1**) or by restricting the correlation calculation to only those variants, amino acids, and

221 HLA types within the classical HLA Class I and II gene bodies (and thus more likely to
222 have functional effect; $p = 0.74$, **Table 1**).

223

224 Until this point, we had established a null distribution by permuting mate pairs and
225 calculating genetic similarity. To generate an alternative null model for comparison, we
226 randomly sampled 10,000 regions from the genome that either matched the MHC by size
227 (i.e., total span of the region) or by number of variants contained within the region
228 (regardless of the total linear span of the region capturing those markers). For each
229 permutation, we randomly selected the region, computed Q_c (averaged across the 239
230 true mate-pairs) and counted the number of times the mean Q_c was as or more
231 dissimilar than that observed in the MHC. We observed no statistically significant
232 difference, after accounting for multiple testing, when selecting regions based on
233 genomic size or total number of markers in the region, after accounting for multiple
234 testing (one-sided $p = 0.08$ and 0.02 , respectively).

235

236

237

238 **Discussion**

239 Using the whole-genome sequencing data of 239 mate pairs, we have performed, to our
240 knowledge, the most comprehensive investigation of MHC-dependent human mate
241 selection to date. The Genome of the Netherlands resource provided both an increased
242 sample size compared to previous efforts [17,29] and high density genetic variation
243 data, allowing for analyses of rare variants, indels, and imputed HLA types. However,
244 despite the size and genomic resolution of the data, our results indicate no evidence for
245 MHC-dependent mate selection in humans. We performed further analyses to investigate
246 the potential effects of geographical clustering of rare variants [30,35], but the results
247 left our results and interpretation unchanged.

248

249 Notably, our results are inconsistent with an initial investigation of MHC-dependent mate
250 selection using genome-wide genetic variation data [17]. Though these previous findings
251 do not align with our own, the initial report of MHC-dependent mate selection in humans
252 was likely too small ($N = 60$) to draw conclusive results. Further, potential confounders,
253 including cryptic relatedness and inbreeding amongst the studied samples, along with a
254 lack of multiple testing correction, all likely contributed to this initial positive finding,
255 subsequently contradicted in follow-up analyses of the same samples [29]. By
256 interrogating a larger sample size, more stringently removing samples for relatedness
257 and inbreeding, and performing analyses that account for potential population
258 stratification, we believe our results provide more robust information as to whether mate
259 selection in humans is influenced, at least in part, by individuals' genetic composition
260 across the MHC. Additionally, our results are consistent with investigation of MHC-
261 dependent mate selection using HLA types in similarly-sized sample sets [23,24].

262

263 While our results indicate that human mate selection is independent of genetic variation
264 in the MHC, a number of studies examining genetic variation and complex traits have
265 found a plethora of positive evidence for assortative mating in humans based on non-

266 MHC genetic factors. Previous studies have shown that human mate choice is associated
267 to quantitative features (such as height) [38], to socioeconomic factors and risk for
268 multifactorial disease [39–41]. A recent analysis in > 24,000 mate pairs, drawn from a
269 number of cohorts including the UK Biobank [42] and 23andMe, focused on genomic loci
270 associated to a number of multifactorial traits and found significant correlation between
271 spouses at loci associated to height and body mass index [43]. By building a genetic
272 predictor in one member of a spousal couple and applying it in the second member, the
273 study also revealed varying degrees of spousal correlation at loci associated to waist-to-
274 hip ratio, educational attainment, and blood pressure [43] in 7,780 couples from the UK
275 Biobank. These correlations represent only a small slice of the numerous factors — both
276 genetic and environmental — that contribute to mate selection in the human population.
277 Importantly, however, these observations are correlative; the extent to which these
278 associations are potentially causative remains to be explored.

279
280 Though our analysis offers several improvements over previous analyses examining
281 MHC-dependent mate selection, several limitations remain. First, as highlighted by the
282 assortative mating studies discussed above, our sample size may not be large enough to
283 detect a more modest signal for MHC-dependent mate selection, if such a phenomenon
284 exists. Mate selection is likely influenced by a host of hundreds, if not thousands, of
285 factors, all of which likely have modest effect. Therefore, analysis of 239 samples may
286 not be sufficiently well powered to detect such an effect. Further, while we have used
287 permutations of mate pairs to establish a null distribution to which we can compare true
288 mate-pair genetic similarity, this distribution may not be sufficiently informative to
289 detect MHC-dependent effects. Indeed, the authors of the initial analyses [17] reported
290 similar difficulties establishing a null comparator: they sought to additionally use
291 genome-wide genetic similarity as a basis of comparison for MHC similarity, but observed
292 higher genome-wide similarity in YRI samples compared to the CEU [17]. Given the
293 uniqueness of the MHC, from its gene density and extensive linkage disequilibrium to its

294 high genetic diversity, finding a genomic region with similar properties to use as a null
295 comparator is essentially impossible; permutations of real mate pairs into random pairs,
296 while not ideal, is likely the best null distribution for this experiment. Additionally, our
297 analysis only examines one ancestral population. Analyses extended into other (non-
298 European) samples may result in different findings.

299

300 Untested here is the hypothesis that preferential mating may favour specific
301 combinations of HLA alleles that collectively result in an 'optimal' number of antigens
302 that can be presented to T cell receptors. Previous studies indicate that this phenomenon
303 may occur, specifically across Class I classical HLA genes [44], and may provide an
304 alternative mechanism for MHC-mediated mate selection. Given the number of HLA allele
305 combinations that would need to be constructed and analyzed to test such a hypothesis,
306 power (after multiple test correction) would be vanishingly small. We therefore have not
307 tested this specific hypothesis. However, additional information regarding gene function
308 may make testing this hypothesis feasible in the future.

309

310 Despite these limitations, our analysis represents an improved investigation of MHC-
311 dependent mate selection, through interrogated sample size as well as in the spectrum
312 of genetic variation tested. Our data indicate no MHC-mediated preferential mating
313 patterns in our European-ancestry sample. While MHC-mediated preferential mating has
314 been reported in non-human animal models, such a mechanism in humans is either
315 absent or may be one of many subtle contributors to mating patterns and behaviours.

316

317 **Materials and methods**

318

319 **Code and data release**

320 Individual-level data generated by the Genome of the Netherlands Project can be
321 accessed through an application, available here: <http://www.nlgenome.nl/>. We provide
322 code for this project at the following GitHub repository: [https://github.com/mcretu-](https://github.com/mcretu-umcu/matingPermutations)
323 [umcu/matingPermutations](https://github.com/mcretu-umcu/matingPermutations).

324

325 **Ethics Statement**

326 All participants provided written informed consent as part of the Genome of the
327 Netherlands project (<http://www.nlgenome.nl/>), and each biobank was approved by
328 their respective institutional review board (IRB).

329

330 **Quality control of HapMap and Genome of the Netherlands data**

331 Related samples, by definition, are more likely to share more genetic variation compared
332 to two unrelated individuals. To ensure that relatedness was not confounding our
333 analyses, we performed basic quality control (QC) in the CEU, YRI and Genome of the
334 Netherlands (GoNL) sample sets separately. The initial HapMap 2 analysis [17] filtered
335 related couples by looking at the normalized Qc measure and defining outliers. We used
336 the identity-by-descent (IBD) estimates, computed with Plink 1.9 [45] using the --
337 genome command. Though this approach differs from the initial analysis, using IBD
338 estimates are an established means for identifying related samples using genetic
339 variation data.

340

341 To estimate relatedness, we first used Plink 1.9 to assemble a set of high-quality SNPs
342 with minor allele frequency (MAF) > 10% and genotyping missingness < 0.1%. We
343 pruned this set of SNPs at a linkage disequilibrium (r^2) threshold of 0.2. Additionally, we

344 removed SNPs in the MHC, lactase (*LCT*) locus on chromosome 2, and in the inversions
345 on chromosomes 8 and 17 (genomic coordinates in **Supplementary Table 1**). We
346 calculated relatedness (--genome in Plink) across all individuals in the CEU and YRI mate
347 pairs. We discarded three mate pairs (N = 6 samples) from the CEU sample and three
348 mate pairs (N = 6 samples) from the YRI sample. We defined relatedness as π -hat >
349 0.05 (i.e., shared 1/20th of the genome), close to the 1/22nd threshold used by Derti *et*
350 *al.* [29]. Our filtering produced nearly identical results to the initial analyses
351 (Supplementary Text S2 of [29]). Due to our slightly more stringent cutoff threshold, we
352 additionally exclude the related pair of samples NA12892 and NA06994.

353

354 We filtered for relatedness in GoNL in an identical manner. We used a more stringent
355 cryptic relatedness threshold of π -hat > 0.03125, corresponding to 5th-degree
356 relatives. We discarded 9 couples from our analysis, leaving 239 QC-passing mate pairs.

357

358 **Calculating genetic similarity in mate pairs**

359 We define genetic similarity across a mate pair (called Q_c , per the initial report [17]) as
360 the proportion of variants that are identical across a pair of individuals. Homozygous
361 genotypes comprised of the same alleles (e.g., AA in sample 1 and AA in sample 2) are
362 considered 100% similar; heterozygous genotypes (e.g., AB in both samples) are
363 considered 50% similar, as they could have either the same or opposite phase; and all
364 other combinations are considered 0% similar.

365

366 We note that in the initial report [17], genetic similarity was defined as: $R = (Q_c -$
367 $Q_m)/(1 - Q_m)$, where Q_m is the average genetic similarity across all possible mate-pairs
368 (real and permuted) that can be constructed in the sample. We note that the R measure
369 is a linear transformation of Q_c measure, as Q_m is a constant for the analyzed sample.
370 Further, Q_m is not an unbiased estimate of the average genetic similarity within random
371 mate-pairs for two reasons: (1) because it includes both real mate-pairs and female-
372 male pairs constructed by selecting two random individuals in the dataset; and (2)

373 because the sample pairs over which Q_m is averaged are not independent (i.e., the
374 same individual is paired with all possible matches and thus considered multiple times
375 when computing Q_m). We therefore perform all our analyses using only the Q_c measure
376 of genetic similarity.

377

378 **Replicating the original HapMap analysis**

379 The HapMap 2 genotyping data is publicly available [27,32,33] and includes a total of
380 3,965,296 single nucleotide polymorphisms (SNPs). We extracted the MHC region (29.7
381 - 33.3Mb on chromosome 6, build hg18, as defined in the original analysis) from each
382 population separately: people of Northern and Western European ancestry (the CEU) and
383 Yorubans from Ibadan, Nigeria (YRI). We performed these analyses in 27 CEU mate-
384 pairs and 27 YRI mate-pairs, after filtering on sample relatedness (see *Quality Control of*
385 *HapMap and Genome of the Netherlands Data*).

386

387 ***Evaluating significance of genetic similarity in true mate-pairs***

388 To evaluate whether genetic (dis)similarity in mate-pairs was significantly different than
389 genetic similarity between two random individuals, we performed a permutation
390 analysis. Specifically, we created 'null' (i.e., non-real) male-female pairs by randomly
391 permuting the individuals in the true mate-pairs. Within any single permutation, we
392 allowed for at most 1 real couple to enable faster sampling of random mate-pairs. We
393 performed a total 1,000,000 permutations to generate a null distribution (**Figures 1** and
394 **2**). Finally, we count the number of permutations that yield an average Q_c that is the
395 same or lower than the Q_c measured in the true mate-pairs. The total number of such
396 permutations divided by 1,000,000 is the exact p-value of the test. This permutation
397 scheme was used to evaluate the significance of Q_c as measured in common variants, all
398 variants, and imputed HLA variants.

399

400 **Analysis of mate-pairs in the Genome of the Netherlands (GoNL)**

401 **data**

402 We repeated the same analysis in the Genome of the Netherlands data (GoNL), in the
403 239 mate-pairs that passed quality control. In the GoNL data, we estimated Q_c in three
404 sets of variants (**Table 1**): common biallelic variants only, all available single nucleotide
405 variants regardless of frequency, and in all available variants (including insertions and
406 deletions). For a fourth set of variants - imputed HLA variation - we measured genetic
407 similarity using Pearson's correlation (r), as the imputed variation data was phased and
408 left no ambiguity as to how heterozygous genotypes correlated (e.g., the difference
409 between observing the AB genotype in Sample 1 and the AB genotype in Sample 2; or
410 observing the AB genotype in Sample 1 and the BA genotype in Sample 2). To evaluate
411 the significance of Q_c in true mate-pairs, we used the identical permutation scheme as
412 used in the HapMap analysis and described above.

413

414 ***HLA imputation***

415 We use SNP2HLA (<http://software.broadinstitute.org/mpg/snp2hla/>) [31] and a
416 reference panel built from HLA typing performed in the Type 1 Diabetes Genetics
417 Consortium (T1DGC) (containing 8,961 markers) [31] to impute SNPs, HLA types and
418 amino acid substitutions across 8 classical HLA loci. For imputation, 3,256 SNPs in GoNL
419 overlap the T1DGC reference panel data. After the MHC imputation was complete, we
420 first performed quality control, removing samples where the total number of imputed
421 alleles is > 2.5 (introduced by imprecision in the imputation algorithm) and removing all
422 variants for which the imputation quality ('info') metric is < 0.8 .

423

424 ***Correcting for population structure in the GoNL samples***

425 As the Dutch samples are drawn from 11 of the 12 provinces in the Netherlands, subtle
426 population structure can be observed in both common and rare variants [30]. Analysis in
427 the original GoNL effort indicated that the first two principal components reveal a subtle

428 north-to-south gradient, and analysis of rarer (so-called “ f_2 ”) variants (two alleles
429 appearing in the entire dataset) indicate strong clustering within geographical regions
430 (north, center, and south, as inferred by IBD analyses) [30]. We thus sought to explore
431 whether population structure, either across the country or by province, may be
432 confounding a potential signal for MHC-dependent mate selection. To do this, we used
433 principal component analysis as well as province-specific analyses.

434

435 Genetic PCs are calculated on an individual basis and are an alternative means of
436 unravelling genetic ancestral clustering between individuals. We first needed to collapse
437 individual-level PC loadings into a single value that represented a single mate-pair. We
438 call this collapsed PC the ‘mate-pair PC’ (PC_{mp}). Assume that the PC1 loading for a
439 female in a given mate-pair is denoted $PC1_f$, and PC1 loading for the male in that mate-
440 pair is denoted $PC1_m$, then $PC1_{mp}$ (continuing up to PC ‘n’) is defined as follows:

441

$$442 \quad PC1_{mp} = (PC1_f - PC1_m)^2$$

443 ...

$$444 \quad PCn_{mp} = (PCn_f - PCn_m)^2$$

445

446 In this way, we used the PCs of the GoNL individuals to obtain, for each (real or
447 permuted) pair of individuals, a PC_{mp} value that is equal to 0 if the loadings of the two
448 individuals in a pair are identical for a given PC, or becomes increasingly large as the two
449 samples’ loadings on a particular PC diverge.

450

451 We then used the mate-pairs of one random permutation of the 239 mate-pairs in GoNL
452 to train a linear regression model that approximates the genetic similarity between two
453 individuals (Q_c), using the mate-pair PCs as defined above:

454

$$455 \quad Q_{Chat} \sim PC1_{mp} + PC2_{mp} + \dots + PC10_{mp}$$

456

457 Q_{chat} estimates the genetic similarity explained by the first 10 PCs for all mate-pairs (real
458 or permuted) as well as residuals (Q_{res}) from this regression. If there is preferential
459 mating among the true mate-pairs in GoNL, the residuals of this regression model should
460 be systematically different compared to residuals from randomly-assigned male-female
461 pairs. We performed the same initial permutation analysis, on the whole set of 239 true
462 mate-pairs, but using Q_{res} (instead of Q_{c}) as a measure of genetic similarity adjusted
463 for population stratification. We then compared where the average Q_{res} across the 239
464 true mate-pairs falls within the distribution of average Q_{res} across 239 randomly
465 generated male-female pairs.

466

467 **Genetic dissimilarity in non-MHC regions**

468 In addition to permuting mate-pairs to establish a null distribution for Q_{c} , we also wanted to establish
469 a null distribution of Q_{c} by randomly sampling regions from the genome that were matched to the
470 MHC based on different characteristics. Because the MHC is an extremely unique genomic region —
471 in gene density, in span of linkage disequilibrium, and in genetic variability — it is nearly impossible to
472 identify regions of the genome that behave identically to the MHC. To identify genomically similar
473 regions to the MHC from which we could construct a null distribution for Q_{c} , we identified regions that
474 either (1) were the same genomic span as the MHC (~3.6 Mb), or (2) contained approximately
475 the same number of markers (~40k), regardless of the linear span of that window. For
476 each criterion (SNP density or span), we randomly sampled 10,000 regions from the
477 genome and computed average Q_{c} across all 239 true mate-pairs, for each region; we
478 compared these distributions to Q_{c} calculated in true mate-pairs across the MHC.

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482 **Acknowledgements**

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484 The Genome of the Netherlands Consortium (<http://www.nlgenome.nl/>) generated and
485 analyzed the whole-genome sequencing data analyzed here. A complete list of the
486 Genome of the Netherlands members and affiliations can be found here:
487 http://www.nlgenome.nl/?page_id=28.

488

489 We thank Paul IW de Bakker for supporting MCS with funding from VIDI grant 91712354
490 from the Dutch Organization for Scientific Research (Nederlandse Organisatie voor
491 Wetenschappelijk Onderzoek (NWO) - ZonMw) and for his critical review of the
492 manuscript.

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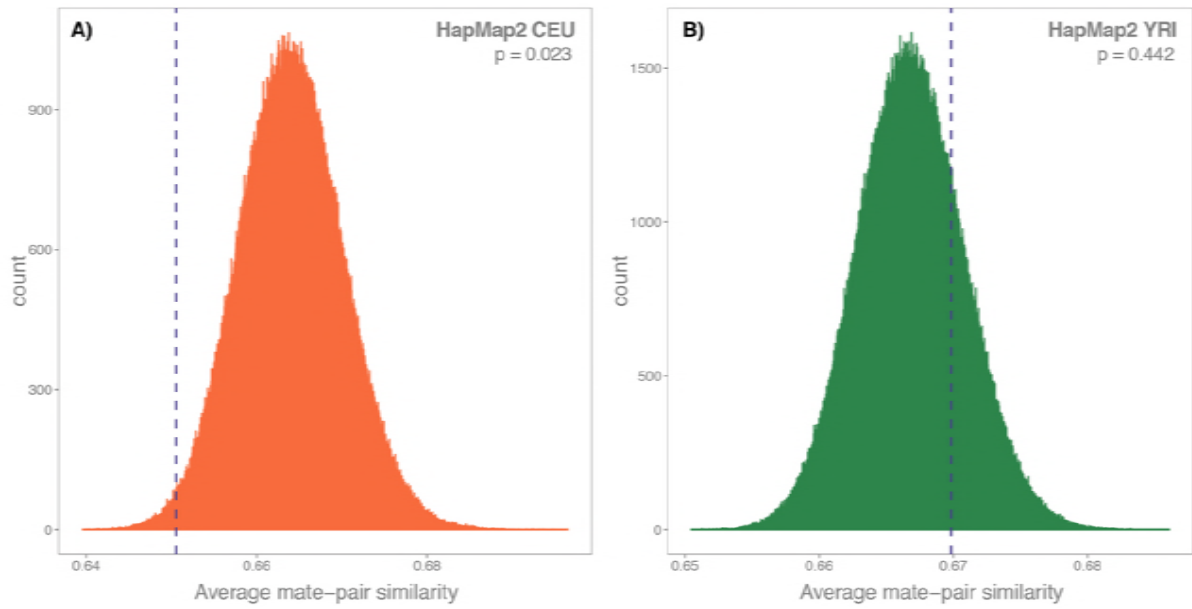
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617 **Figures and Tables**

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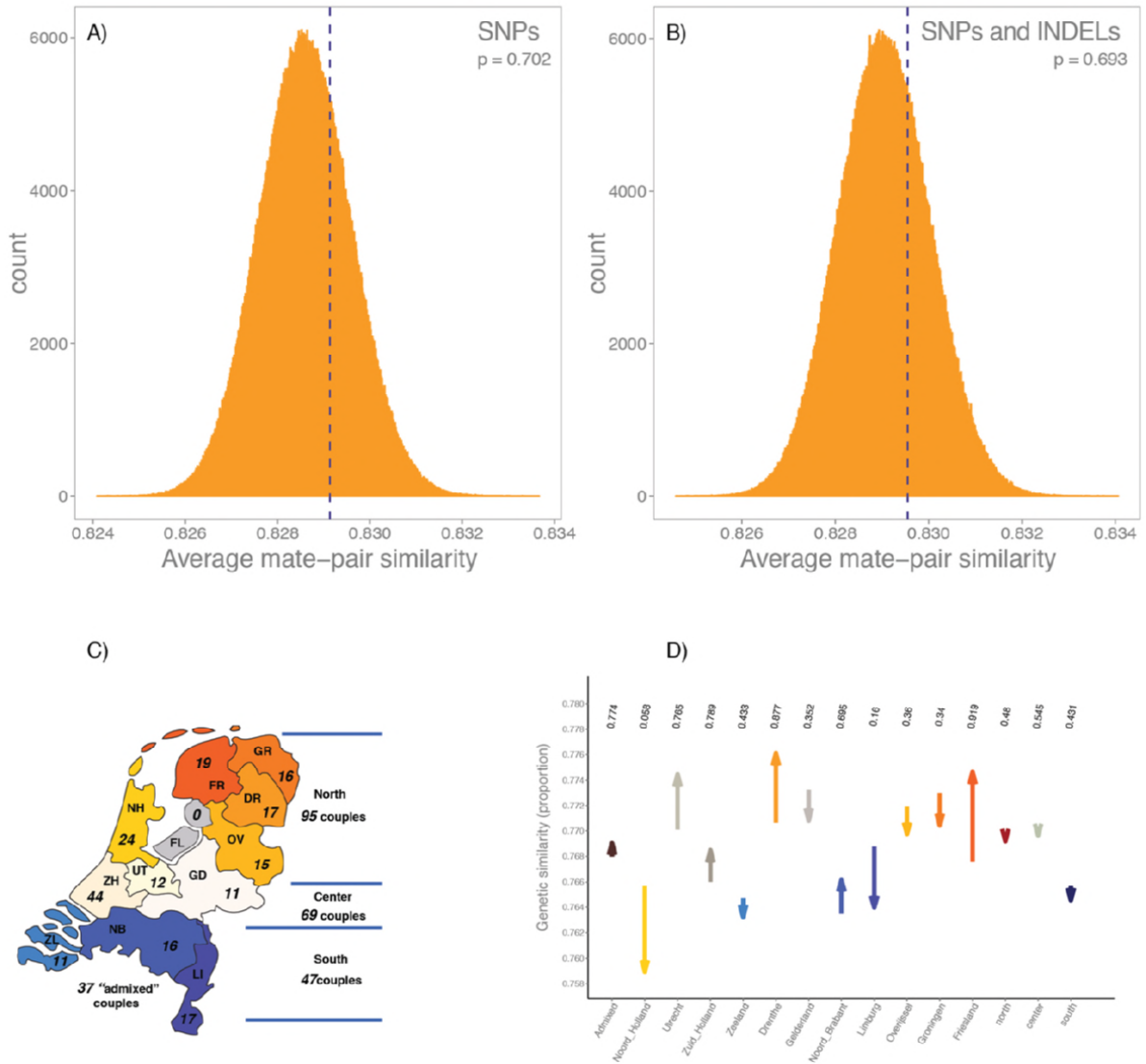
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622 **Figure 1**

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625 **Figure 2**

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627 **Figure 1 | Genetic similarity across mate pairs in the HapMap 2 data.** The distributions

628 represent the null distribution of average MHC similarity (Q_c), across randomly permuted mate pairs from each of

629 the HapMap 2 populations tested (CEU: European samples of Northern and Western descent, orange; YRI:

630 Yorubans in Ibadan Nigeria, green). The average MHC similarity in true mate pairs is marked by the blue dotted

631 line. All p-values are based on 1,000,000 permutations and delta Q_c (ΔQ_c) is the difference between the average

632 real-couple similarity and the average of the distribution of random mate-pair permutations. **(A)** Permutation

633 of the 27 QC-passing HapMap 2 CEU couples. $\Delta Q_c = -0.013$, 2-sided $p = 0.023$. **(B)** Permutations of

634 the 27 QC-passing HapMap 2 YRI couples. $\Delta Q_c = 0.003$, 2-sided $p = 0.442$.

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636

637 **Figure 2 | Genetic similarity across the MHC for 239 Dutch-ancestry mate-pairs.** Panels

638 **(A)** and **(B)** show the null distribution (histograms) of average mate-pair genetic similarity of

639 permuted (i.e., non-real) male-female pairs. We performed a total of 1,000,000 permutations to

640 generate the distribution. The average genetic similarity across 239 real mate pairs is represented

641 with a blue vertical dotted line. **(A)** Genetic similarity measured across all biallelic variants within

642 the MHC ($p = 0.702$). **(B)** Genetic similarity measured across all biallelic variants and

643 insertions/deletions (indels) in the MHC ($p = 0.693$). **(C)** The GoNL samples were drawn from 11

644 of the 12 Dutch provinces. Here, we indicate the number of true mate-pairs available for analysis

645 where both members of the mate-pair come from the same geographic region. These three

646 geographic regions (north, center, and south) are derived from previously-performed population

647 genetic analyses of the GoNL data. **(D)** Genetic similarity of mate-pairs, split by province. The

648 arrows start at the average genetic similarity of permuted (i.e., null) mate pairs and stops at the

649 average genetic similarity across true mate-pairs. Corresponding, one sided p-values for the

650 genetic dissimilarity within couples are marked above.

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654 **Supporting information Legends:**

655 **SupplementaryMaterials.docx** : File containing Supplementary Figures 1 through 5, referenced
656 in the main text.

657 **CoverletterPlosGenCretuStancu.docx** : Cover letter to the PLoS Genetics editors

658

659 **Tables:**

Sample	Number of mate-pairs	Data type	Variant type(s)	Variant filters	Variant count	ΔQ_c	p-value
CEU	27	Genotyping (HapMap)	SNVs	MAF > 5%	6,247	-0.0130	0.023
YRI	27			5,773	0.0030	0.442	
GoNL	239	Sequencing	SNVs	None	60,339	0.0005	0.702
				HapMap2 sites	8,573	0.0004	0.513
				MAF > 0.5%	44,088	0.0007	0.703
				MAF > 5%	31,145	0.0001	0.709
				Extended MHC	36,413	0.0004	0.696
				SNVs + indels	63,357	0.0004	0.693
		HLA imputation	SNVs	$r^2 > 0.8$	8,290	-	0.480
			HLA alleles Amino acids	Genic markers $r^2 > 0.8$	2,452	-	0.740

660

661 **Table 1 | Samples and variants used in analysis.** To investigate whether mate selection is
662 MHC-dependent, we analyzed three sample groups: Utah residents with Northern and Western
663 European ancestry (CEU); Yorubans from Ibadan, Nigeria (YRI); and mate-pairs in the Genome of
664 the Netherlands (GoNL) project. The number of mate pairs indicates the number of pairs available

665 after sample quality control. We performed our analysis in common polymorphisms (minor allele
666 frequency (MAF) > 0.05) or common- and low-frequency single nucleotide variants (SNVs, with
667 MAF > 0.5%), as well as including indel variation, where available. For imputed data, we kept only
668 well-imputed data, based on the Beagle imputation quality metric ($r^2 > 0.8$). We additionally
669 restricted the set of variants to only variants within the classical HLA genes including amino acid
670 substitutions, single nucleotide polymorphisms (SNP), insertions and/or deletions (indels) and
671 classical HLA-types ('genic markers').

672